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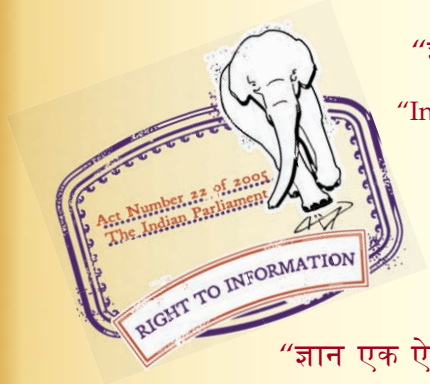

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IS : 6261 - 1971
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Indian Standard

METHODS OF ANALYSIS FOR DETECTION OF INSECT AND RODENT CONTAMINATION IN GRAINS AND MILLED PRODUCTS

(First Reprint JUNE 1988)

UDC 664.7:543.9—078

BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

Gr 7

May 1972

Indian Standard

METHODS OF ANALYSIS FOR DETECTION OF INSECT AND RODENT CONTAMINATION IN GRAINS AND MILLED PRODUCTS

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Indian Standard

METHODS OF ANALYSIS FOR DETECTION OF INSECT AND RODENT CONTAMINATION IN GRAINS AND MILLED PRODUCTS

0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 10 June 1971, after the draft finalized by the Foodgrains, Foodgrain Products and Edible Oilseed Flours Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 Food sanitation has been recognized as an important part of quality control throughout the world and has an increasing significance in India today. The foods infested by insects undergo some chemical changes and alter their flavour and nutritive value. Such foods, besides being unacceptable to the consumer and the industry, are also reported to cause certain diseases in animals and human beings and result in malnutrition among children.

0.3 Presence of these insect and rodent contaminants affords an indication of poor sanitation in storage or in the processing units and enables adoption of timely prophylactic or corrective measures. In order to assess the degree of insect and rodent contamination and ultimately the quality of grains and milled products, suitable parameters are to be employed. Of the various parameters insect fragment count, uric acid determination, carbon dioxide evolution, direct insect count, determination of rodent excreta and hair are fairly reliable and could be employed.

0.4 This standard, based on the national and international practices is, therefore, being issued to ensure adoption of uniform methods of analysis for insect and rodent contamination throughout the country, thereby facilitating the interpretation and comparison of results.

0.5 In the preparation of this standard considerable assistance has been derived from BS 1756 : Part 3 : 1965 ' Methods for the sampling and analysis of flue gases; Part 3 Analysis by the Haldane apparatus ' issued by the British Standards Institution.

0.6 In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS : 2-1960*.

*Rules for rounding off numerical values (*revised*).

1. SCOPE

1.1 This standard prescribes methods of analysis for detection of insect and rodent contamination in grains and milled products.

2. SAMPLING

2.1 The method of sampling of grains and milled products for analysis shall be in accordance with IS : 5315-1969*.

3. QUALITY OF REAGENTS

3.1 Unless specified otherwise, pure chemicals and distilled water (*see* IS : 1070-1960†) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the experimental results.

4. VISUAL EXAMINATION

4.1 Procedure — Spread a weighed quantity of the sample of grain or milled product over a light coloured surface and visually examine the sample with the help of a hand lens (10 ×) for rodent pellets and insect larvae, pupae and adults. Examine the grains for insect emergence holes in kernels. Verify the rodent pellets by crushing them in a small quantity of mineral oil or glycerine and location of rodent hairs on them.

4.2 Estimation — Estimate the percentage by weight of kernels having insect emergence holes and number of live or dead insects, and rodent pellets for the weight of the sample taken.

5. SIEVE TEST

5.1 Procedure — Sieve a weighed quantity of the sample using 1·40- or 1·70-mm IS Sieve or equivalent.

5.1.1 Record live or dead insects and identify them, if possible. Express as number of insects (larvae, pupae or adults) for the weight of the sample taken. The test report should indicate the sieve size used.

6. DETECTION OF HIDDEN INSECT INFESTATION IN GRAINS

6.0 Methods — Two methods, namely the staining method and the cracking floatation method, are used for detecting hidden insect infestation. These methods are described in **6.1** and **6.2**.

*Methods of sampling of milled cereals and pulses products.

†Specification for water, distilled quality (*revised*).

6.1 Staining Method

6.1.1 *Reagents*

6.1.1.1 *Acid fuchsin* — 0·5 g.

6.1.1.2 *Acetic acid glacial solution* — Measure 50 ml of the acid and mix in 950 ml of distilled water.

6.1.1.3 *Distilled water*

6.1.1.4 *Preparation of the dye solution* — Add the acid fuchsin to the glacial acetic acid solution and store in a clean bottle.

NOTE — This dye solution can be stored for a long time and may be used repeatedly until it becomes murky.

6.1.1.5 *Iodine solution* — prepared by dissolving 10 g of potassium iodide in some water in a flask with ground glass stopper. Add to this solution 5 g of crystalline iodine and shake until iodine is completely dissolved. Dilute to 500 ml with water.

6.1.1.6 *Potassium hydroxide solution* — 0·5 percent.

6.1.2 *Procedure* — Take a weighed quantity of grain and soak in warm water for 5 minutes. Drain off water using a strainer and cover the grain with acid fuchsin solution (**6.1.1.4**) for 4 to 5 minutes, taking care not to leave the grains longer in the solution, which may result in kernels absorbing excess of solution and making the identification of egg plugs difficult. Pour off the dye solution (retain for future use) and wash grain in tap water to remove excess dye. Examine the kernels to locate the gelatinous egg plugs which stain a deep cherry red. As against this, the feeding punctures, and mechanical injuries stain a lighter colour than the egg plugs.

6.1.2.1 In the case of pulses, the material after being placed on a sieve is immersed in the iodine solution (**6.1.1.5**). Subsequently, the sieve with the seeds is immersed in potassium hydroxide solution. Now take out the sieve from the solution and rinse with cold water. The entry openings of the larvae and the points of attack are stained black by this treatment. Seeds with round black spots on the surface are considered as infested. The examination should be carried out as soon possible, as the discolouration will gradually fade.

6.1.3 Separate and estimate the number of infested grains for the above weight of the sample.

6.2 Cracking Floatation Method

6.2.1 *Apparatus*

6.2.1.1 *Balance*

6.2.1.2 *Sieve* — 1·40-mm IS Sieve or equivalent.

6.2.1.3 *Laboratory grinder* — set at 1·5 mm.

6.2.1.4 *Buchner funnel*

6.2.1.5 *Filtering flasks*

6.2.1.6 *Microscope, binocular* — with 15 × to 30 ×, wide field.

6.2.2 *Reagents*

6.2.2.1 *Alcohol solution* — 60 percent, saturated with any lead-free petrol.

6.2.2.2 *Glycerine water solution* — 1 : 1.

6.2.3 *Procedure* — Mix thoroughly a weighed quantity of grain. Weigh out 100 g and sift through a 1·40-mm IS Sieve to remove any external insects in the sample. Make note of the number of these insects. Grind the sieved sample to crack the grain roughly the size of ¼ kernel of wheat. Transfer the cracked grain to a 1- or 2-litre Wildman trap flask and add about 600 ml of 60 percent alcohol saturated with petrol. Add additional 20 ml of petrol and stir the contents without splashing. Fill the flask with alcohol-petrol solution and allow the material to settle for 15 minutes. Drain off petrol layer and filter through fluted analytical filter paper. Transfer the filter paper to a petri dish to which a few drops of glycerine-water solution have been added. Using the binocular microscope, examine the filter paper for number of whole insects, major insect parts, whole larvae, cast larval skins and larval capsules.

7. DETECTION OF LIVE INSECT INFESTATION IN GRAINS (CARBON DIOXIDE EVOLUTION METHOD)

7.1 Apparatus

7.1.1 *Air-Tight Containers* — of any size that can be conveniently filled. Glass bottles of 500 ml capacity are suitable. Each bottle is closed with a rubber stopper, carrying a tube of 1-2 mm bore which is fitted with a glass stopcock.

7.1.2 *Syringes* — for withdrawing samples of the interstitial air for analysis. The syringe should be completely air-tight and should hold sufficient air for analysis by the gas-analysis apparatus used. All glass syringes of 20 ml capacity are convenient.

7.1.3 *Incubator* — operating at 25°C and controlled to within ±1°C.

7.1.4 *Gas-Analysis Apparatus* — Haldanes apparatus, the details of which are given in **7.1.4.1**. The general arrangement of the apparatus shall be as given in Fig. 1.

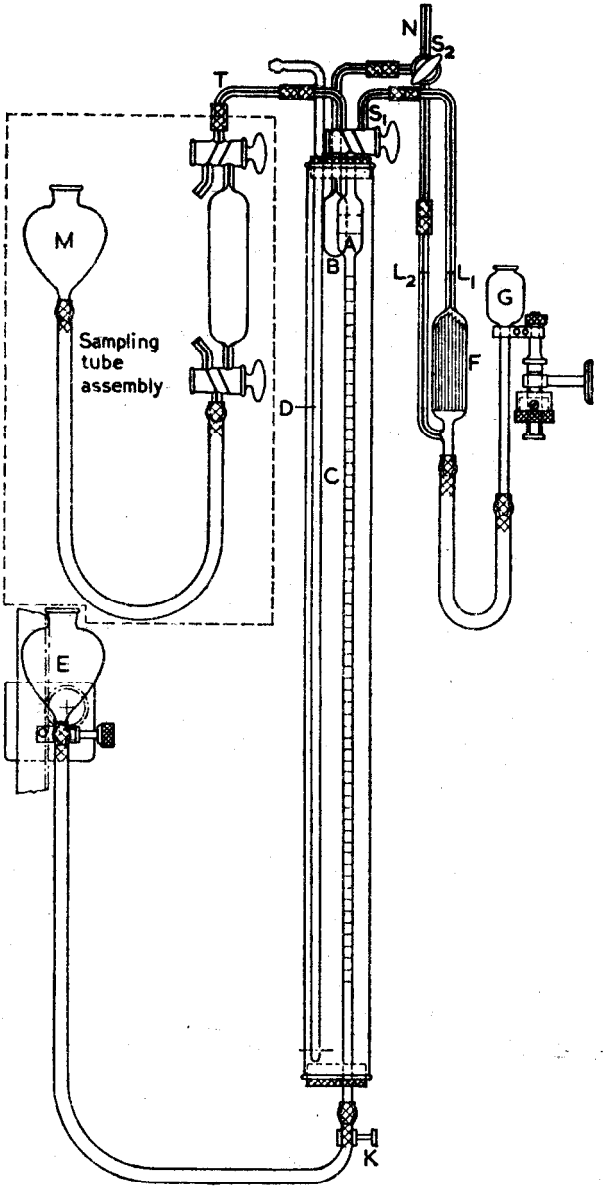


FIG. 1 GENERAL ARRANGEMENT OF THE MODIFIED HALDANE APPARATUS FOR CARBON DIOXIDE ONLY

7.1.4.1 *Components of apparatus*

a) *General* — All glassware shall be constructed of clear glass, shall be as free as possible from striae and other visible defects and shall be well annealed. All open ends of tubes shall be cut off square and flame polished. All capillary tubing shall be not less than 7·0 mm nor more than 8·0 mm external diameter and not less than 1·5 mm nor more than 2·0 mm bore. The stopcocks shall be of the highest quality.

b) *Burette* — The burette *A* shall conform to the dimensions given in Fig. 2.

c) *Compensating bulb* — The compensating bulb *B*, capacity approximately 20 ml, shall conform to the dimensions given in Fig. 3. It shall be made of similar glass to that used for the burette.

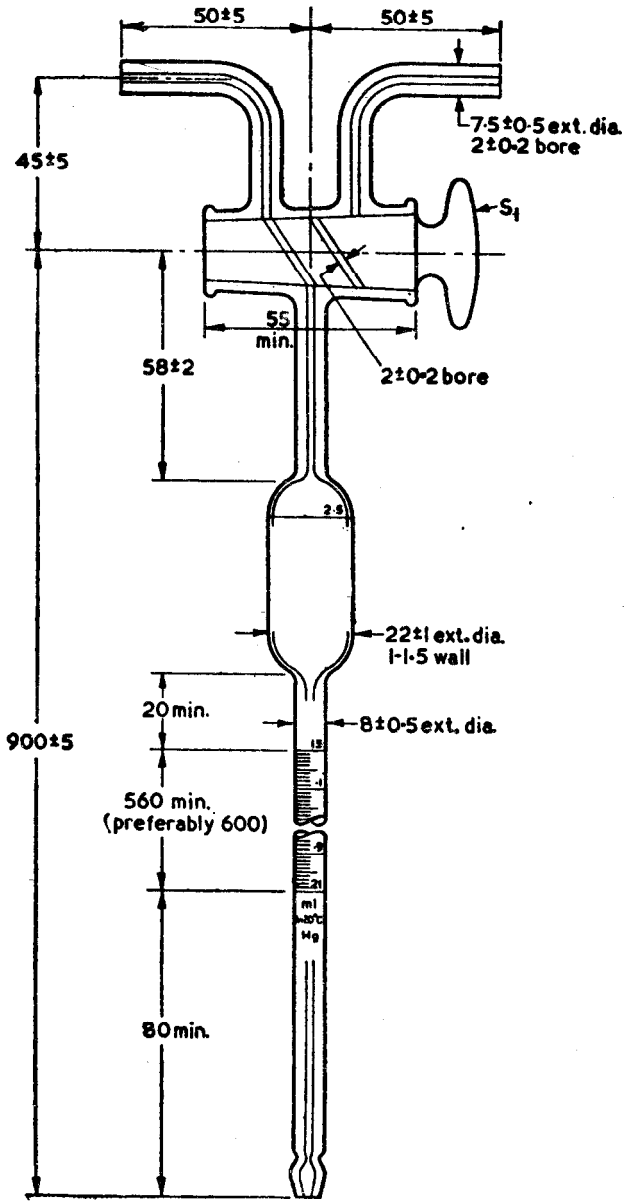
d) *Water jacket and air tube* — The water jacket *C* shall consist of a glass tube conforming to the dimensions given in Fig. 4A. The lower end shall be closed by means of a water-tight rubber bung having a hole bored through it (off-centre) to accommodate the lower end of the burette. The upper end shall be provided with a suitably bored and split rubber bung to accommodate the stem of the compensating bulb, the upper end of the burette and an air tube *D* (Fig. 4 B) to supply the stream of air bubbles to stir the water. This bung shall have an additional hole as an air vent.

e) *Mercury reservoir* — The mercury reservoir *E* for the burette shall be a pear-shaped heavy glass reservoir, capacity approximately 100 ml.

f) *Potash pipette* — The potash pipette *F* for absorbing carbon dioxide shall conform to the dimensions given in Fig. 5. The body of the pipette shall be packed with pieces of glass tubing which approximate in length to that of the body and extend into the upper domed portion; the upper end of each tube shall be cut off obliquely as shown in the figure and the tubes shall be supported by a device, for example, a grid, which also serves to prevent obstruction of the exit.

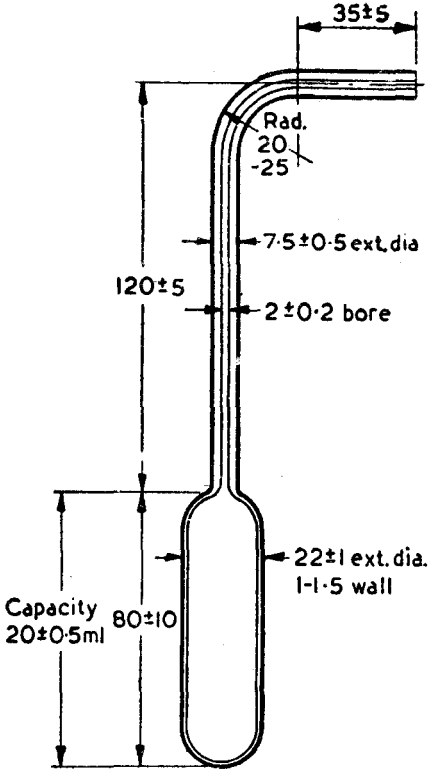
The reference lines L_1 and L_2, one on the capillary tubing above the body of the pipette and the other level with it on the side arm, shall be clear permanent lines of maximum thickness 0·15 mm, completely encircling the tubes and lying in the horizontal plane when the axis of the pipette is vertical.

g) *Potash reservoir* — The potash reservoir *G* shall conform to the dimensions given in Fig. 6.



All dimensions in millimetres.

FIG. 2 BURETTE *A*



All dimensions in millimetres.

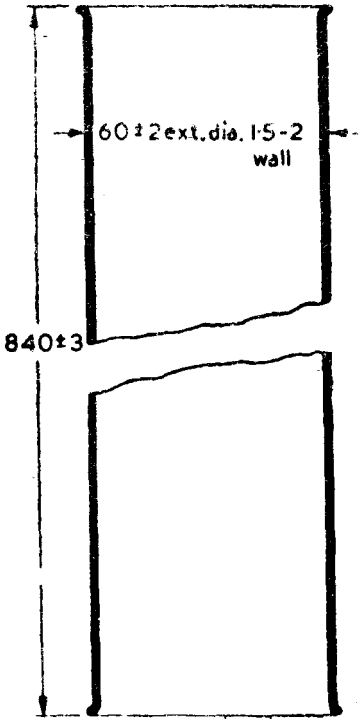
FIG. 3 COMPENSATING BULB *B*

h) *Connecting piece* (*N*) — This component (Fig. 7) connects the potash pipette *F* and the compensating bulb *B*. It shall be manufactured with tubes *X* and *Y* each approximately 75 mm longer than necessary. Before assembly, these tubes are cut off to the required length (*see* **7.1.4.2**) and flame polished.

j) *Sample intake tube* (*T*) — The sample intake tube *T* shall conform to the dimensions given in Fig. 8.

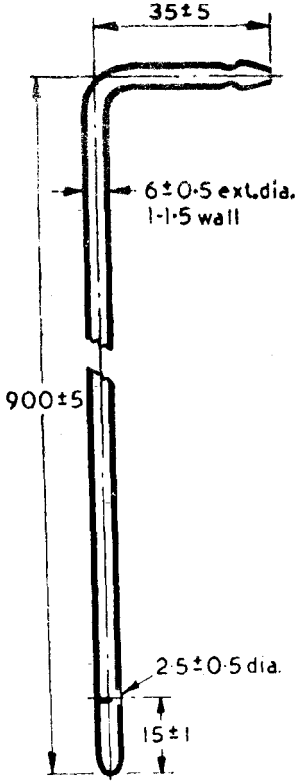
k) *Telescope or magnifier* — This shall be suitably supported to avoid parallax error and shall enable burette readings to be observed to 0·001 ml.

m) *Fine adjustment devices* — These shall enable the positions of the mercury reservoir *E* and the potash reservoir *G* to be set to 0·05 mm. Suitable devices are shown in Fig. 9 and 10.



All dimensions in millimetres.

FIG. 4A WATER JACKET *C*



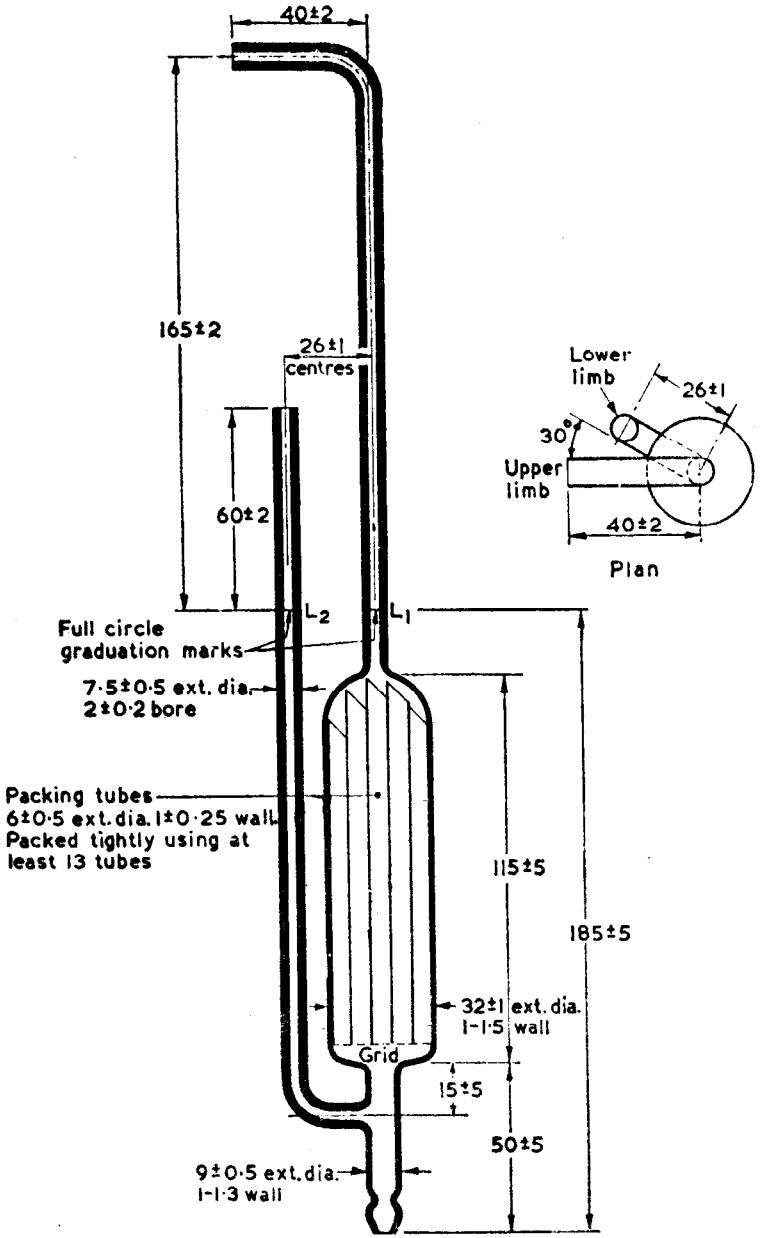
All dimensions in millimetres.

FIG. 4B AIR TUBE *D*

n) *Support* — The apparatus should be mounted on a suitable support, for example, a rigid adjustable framework of rods. The method of mounting should enable the positions of the components to be easily adjusted, thus facilitating the butting together of the various pieces.

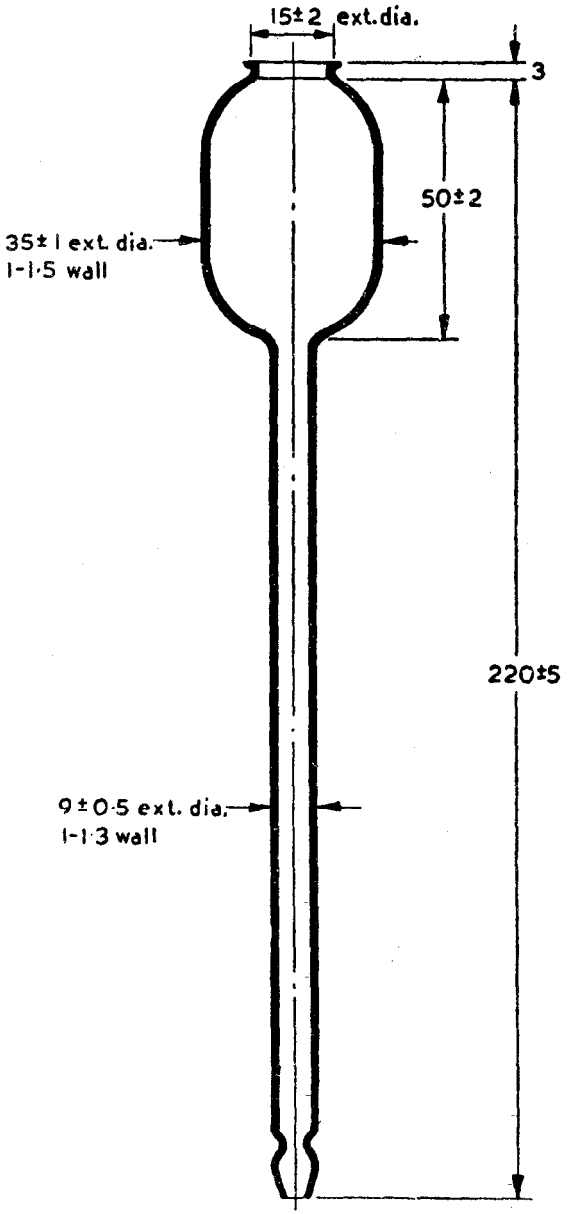
p) *Mercury tray and shields* — The use of these accessories is advisable to keep within bounds any mercury accidentally spilt.

7.1.4.2 *Assembly* — Thoroughly clean the interior of the glass components, using concentrated nitric or sulphuric acid containing a small proportion of chromic acid. Rinse them thoroughly with distilled water and dry by passing clean air through them. Do not use an organic solvent to clean the glassware.



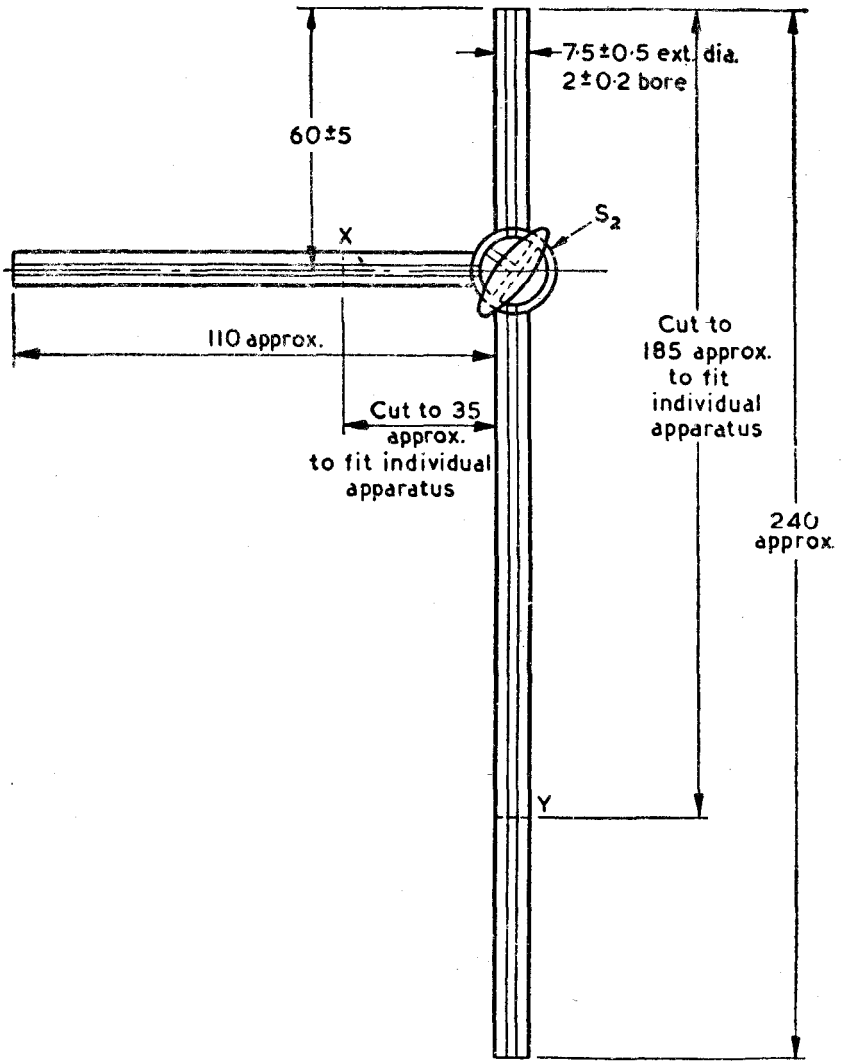
All dimensions in millimetres.

FIG. 5 POTASH PIPETTE *F*



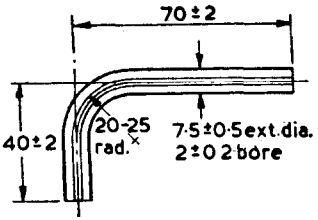
All dimensions in millimetres.

FIG. 6 POTASH RESERVOIR *G*



All dimensions in millimetres.

FIG. 7 CONNECTING PIECE *N*



All dimensions in millimetres.

FIG. 8 SAMPLE INTAKE TUBE *T*

Place 1 ml of the 0·5 percent sulphuric acid in the compensating bulb *B* and assemble the apparatus as shown in Fig. 1. Do not grease the stopcocks at this stage but remove the keys from them. Connect the mercury reservoir *E* to the burette *A* and the potash reservoir *G* to the pipette *F*, using odourless, inert, transparent or translucent 5-mm bore plastic or sulphur-free rubber tubing. For sleeves, use 40-mm lengths of suitable rubber or plastic tubing and ensure that the components are butted together. The last component to be fitted is the connecting piece *N*. Before inserting it, cut off *X* and *Y* to lengths which enable glass-to-glass connections to be made and flame polish the ends. The approximate lengths of these tubes are shown in Fig. 7.

Position the potash reservoir *G* so that the midpoint of the stem is approximately level with the reference marks L_1 and L_2 on the arms of the pipette *F*. Add the appropriate potassium hydroxide reagent to the reservoir until the reagent reaches L_1 and L_2.

Place sufficient mercury in the burette reservoir *E* so that, when the burette is full, a few millilitres of mercury remain in the reservoir. Add the mercury in small quantities and, after each addition, expel any air trapped in the flexible tubing. Leave the burette almost full of mercury.

Grease and insert the keys of stopcocks S_2 (*see* Note).

Grease and insert the key of stopcock S_1 (*see* Note).

Draw into the burette, from *T*, 2-3 ml of the 0·05 percent sulphuric acid. Lower and raise the mercury reservoir *E* several times so that the sulphuric acid thoroughly wets the interior of the burette.

Lower the reservoir so that the mercury meniscus in the burette is below the 21-ml graduation. Nearly close the screw clip *K* to restrict mercury flow. Raise the reservoir and allow mercury to flow slowly into the burette, to fill it and eject surplus sulphuric acid through *T*. The amount of acid remaining should be insufficient to form a layer which completely covers the mercury in the stem.

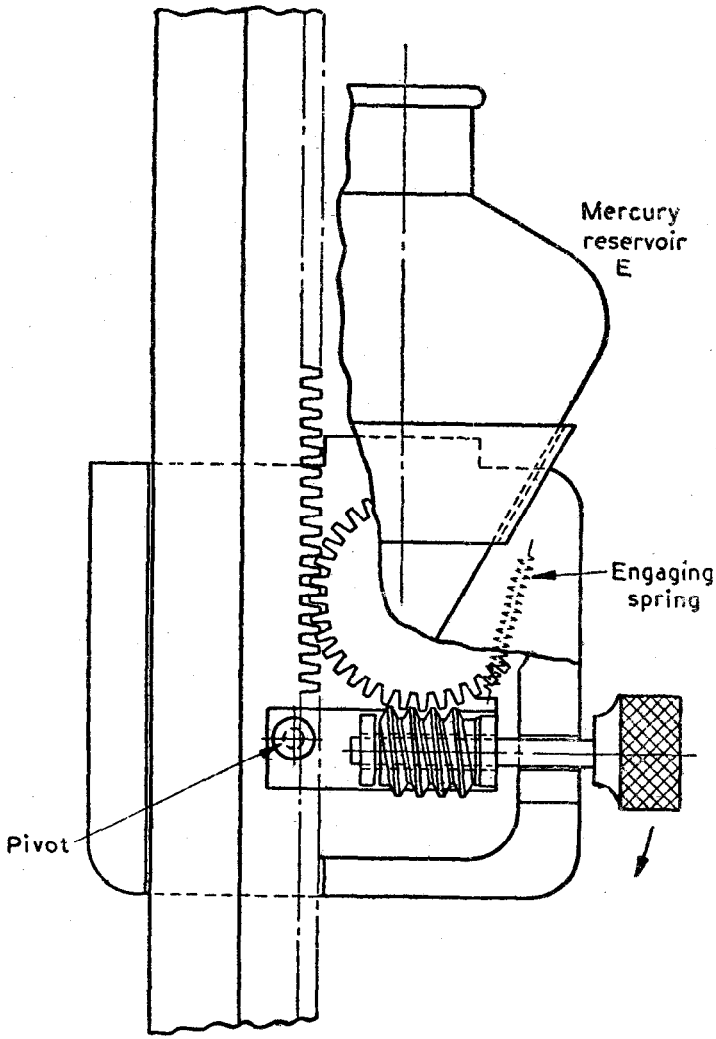


FIG. 9 SUITABLE LEVELLING DEVICE FOR MERCURY RESERVOIR *E*

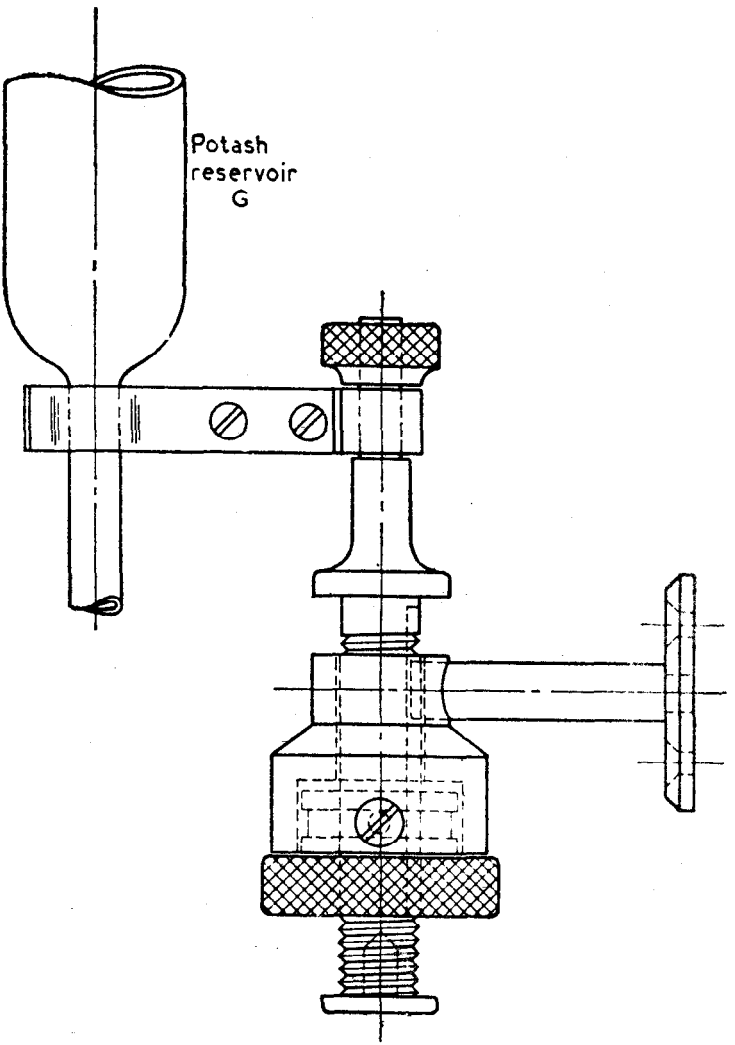


FIG. 10 SUITABLE LEVELLING DEVICE FOR POTASH RESERVOIR *G*

Place sufficient water in the jacket C to cover completely the bulbs of the burette and compensator. Connect the air tube D to a low pressure compressed air source and keep the water in the jacket stirred continuously by means of a steady slow stream of bubbles.

NOTE — Use the minimum quantity of a good quality stopcock grease free from silicones; do not leave grease in the capillaries or in the bores of the keys.

7.1.4.3 *Checking the apparatus* — Turn stopcock S_1 to open the burette to air and draw about 20 ml of air into the burette A through T. Pass the gas six times between the burette and the potash pipette. With stopcock S_2 open to air, and using the fine adjustment devices for the mercury reservoir E and potash reservoir G, adjust the potash in the arm of the pipette F to L_1. Turn S_2 to isolate the compensating system from the air and to connect the compensating bulb B to the potash pipette. If necessary, readjust the potash to L_1 and L_2 and read the burette.

Pass the gas six times between the burette and the potash pipette, adjust the potash to L_1 and L_2 and read the burette. The reading should not have changed by more than 0·001 ml.

7.1.4.4 For the successful operation of the Haldane apparatus, all taps shall be properly greased and the stopcocks and capillaries free from droplets of mercury and potash. The burette shall be free from grease and dirt and contain sufficient dilute sulphuric acid to wet the walls but insufficient to drain and form a layer covering the mercury in the stem.

Only when gas is being taken into or expelled from the apparatus should mercury be allowed to pass S_1; at no other time should mercury or any reagent be allowed to reach any stopcock. When passing gas to and fro between the burette and a pipette, mercury should not be allowed to reach S_1 and potash should not be allowed to pass beyond L_1.

When gas is being passed between the burette and the potash pipette, stopcock S_2 should be shut temporarily to isolate the pipette from the compensating bulb. This reduces the movement of potash solution in the arm of the pipette connected to the compensator and thus minimizes possible drainage errors.

Whilst setting the level of potash to the appropriate reference mark, the capillary should be gently tapped to eliminate 'sticking', as also should the burette jacket before readings are taken.

Before reading the burette, the compensating bulb is connected to the potash pipette through stopcock S_2. All burette readings shall be made to 0·001 ml, with the burette connected to the potash pipette. During checking or the analysis of a portion of sample, the compensating system shall not be reopened to the atmosphere.

If at any stage volume changes greater than 0·002 ml are obtained, that part of the procedure shall be repeated and, if necessary, the responsible fault located and eradicated. Consistent volume changes not greater than 0·002 ml during the combustion process may be treated as 'blanks' and corrections applied accordingly.

7.2 Reagents

7.2.1 *Mercury* — pure redistilled mercury.

7.2.2 *Saturated Potassium Hydroxide Solution* — Treat 500 g of potassium hydroxide with 250 ml of water and cool to room temperature. If solid potassium hydroxide does not persist, add further small quantities until saturation is achieved. Store in a polythene bottle.

NOTE — Potassium hydroxide which has been crystallized from alcohol is unsuitable.

7.2.3 *'Half-Saturated' Potassium Hydroxide Solution* — Dilute saturated potassium hydroxide solution with an equal volume of water. Store in a polythene bottle.

7.2.4 *Twenty Percent Potassium Hydroxide Solution* — Dissolve 200 g of potassium hydroxide in water and dilute to 1 litre. Store in a polythene bottle.

7.2.5 *Sulphuric Acid (0·5 Percent v/v)* — Pour 10 ml of sulphuric acid ($d = 1{\cdot}84$) into 2 litres of cold water. Add a trace of a wetting agent which has a negligible vapour pressure.

7.3 Procedure

7.3.1 *Removal of Free-Living Insects* — If the main interest is in the hidden stages of development, for example, immature weevils, free-living insects should be removed from the sample, usually by sieving. Record the numbers and stage of each species.

7.3.2 *Pre-incubation* — In areas where the produce is likely to become cold, samples should be kept at or near incubation temperature for about 24 hours before being sealed up for incubation. Use small bags, wide-mouthed jars with open tops, trays, or open tins to contain the samples during this pre-treatment, the object of which is to acclimatize the insects to the higher temperature. To prevent the escape of free-living insects, cover the containers with muslin.

7.3.3 *Airing* — In order to ensure that no carbon dioxide is sealed up with the sample, spread the sample thinly on a tray or sheet of paper and leave for 15 to 30 minutes before transfer to the air-tight containers.

7.3.4 *Determination of Moisture Content* — It is desirable (though not essential if the moisture content is known to be below 14 percent) to

determine the moisture content of the sample immediately before the bulk of the sample is bottled for incubation. Note at this stage whether the sample is visibly mouldy.

7.3.5 *Filling the Bottles* — Leave the bottles unstoppered before use to ensure that they are dry and do not contain abnormal amounts of carbon dioxide. To ensure consistency of packing, and hence of intergranular air space, fill the bottle, shake down by tapping a few times on the table, and make up the level with a few more grains. Put the stopper in place, with the stopcock in the capillary open, and push it well down until it is firmly in contact with the material in the bottle. Close the stopcock.

7.3.6 *Incubation* — Incubate the samples for 24 hours at 25°C. If large bottles are used, time should be allowed for heat to penetrate the mass of material. The extra time required when the sample is originally at a temperature between 10° and 15°C is given by the empirical relation:

$$t = d^2 - 50$$

where

t = extra incubation time, in minutes; and

d = external diameter of (cylindrical) bottle, in cm.

The correction may be neglected if the extra time given by this formula is less than 30 minutes, as it is for all ordinary bottles up to 500 ml capacity.

7.3.7 *Removal of Air for Analysis* — Expel all air from the syringe, connect it to the bottle by a short rubber tube fitted with a clip, open the stopcock and draw air into the syringe. In order to make negligible the error due to the air in the rubber tube diluting the sample, move the piston of the syringe backwards and forwards several times so as to mix the air in the tube thoroughly with all the air in the bottle. Finally, close the stopcock while the piston is held out far enough for the syringe to hold a convenient sample of air, close the clip on the rubber tube and disconnect the rubber tube from the bottle.

7.3.8 *Analysis of the Sample*

7.3.8.1 *Setting the levels* — With the compensating system open to the atmosphere, adjust the potash to L_1 and L_2. Isolate the compensating system from the atmosphere.

7.3.8.2 *Introduction of a portion of the sample* — Connect one of the stopcocks of the sampling tube to the intake tube T. Connect a 250-ml mercury reservoir M to the other stopcock of the sampling tube as shown in Fig. 1. Eject any air trapped between M and the sampling tube through the spare limb of its lower stopcock. The gas to be withdrawn from the sampling tube may then be replaced by mercury without contamination of the remainder of the sample. Raise the mercury reservoir E and fill the burette and tube T with mercury, ejecting the nitrogen and a

few droplets of mercury through the spare limb of the upper stopcock of the sampling tube.

Flush out the burette and intake tube with the sample, discharging the gas used to the atmosphere. Open the sampling tube to the burette, lower the mercury reservoir E until the level of the mercury in the burette is below the 21-ml mark and close clip K. Raise the mercury reservoir M to exert a pressure on the gas in the sampling tube and burette of about 10 mm Hg. Close the upper stopcock of the sampling tube, open clip K and raise the mercury reservoir E until the level of the mercury in the burette is between the 20- ml and 21- ml graduations. Close clip K and momentarily vent the burette to air through the upper stopcock of the sampling tube to blow off the excess sample. Read the burette, close S_1, open clip K and adjust the height of reservoir E until the same burette reading is obtained. Cautiously open stopcock S_1 to connect the burette with the potash pipette. If any sudden movement of the potash mensicus from L_1 occurs, readjust the position of the mercury reservoir E. The total movement of the potash from L_1 and L_2 should not be allowed to exceed 1 cm. Adjust the potash to L_1 and L_2 and read the burette. This is the initial volume of the sample. Pass the gas six times between the burette and the potash pipette, adjust the potash to L_1 and L_2 and read the burette. Repeat the procedure until two successive readings differ by not more than 0·001 ml.

7.4 Interpretation of Results

7.4.1 *General Condition* — Uninfested grain containing less than 15 percent of moisture may produce up to 0·25 percent of carbon dioxide in 24 hours at 25°C, and figures up to 0·3 percent may be considered to give no positive indication of the presence of insects or of substantial amounts of micro-organisms.

A carbon dioxide figure between 0·3 and 0·5 percent indicates a slight insect attack or rather high activity by micro-organisms, probably due to high moisture content. Material giving such a result is probably not suitable for storage for more than one or two months. If confirmation of the result is desired, the sample should be incubated for further 24 hours without airing, in order to double the concentration and hence reduce the errors in the carbon dioxide determination.

Material that gives a carbon dioxide figure between 0·5 and 0·9 percent should be kept under close observation and the test repeated at fortnightly intervals.

A carbon dioxide figure of 1 percent may be taken as the lower limit of the dangerous condition. Material which gives a carbon dioxide figure of 1 percent or higher is to be regarded as highly unsuitable for storage.

The above figures apply to all varieties of wheat, peas, split peas, haricot beans, polished rice and similar small, huskless, hard grains; for these commodities the effect of variations in intergranular air space is negligible. For other commodities it is necessary to make a correction for the characteristic volume of their air spaces, and a number of correction factors are given below. (The observed carbon dioxide figure is to be multiplied by the correction factor.)

Product	*Correction Factor*
Linseed	0·89
Small yellow maize	1·05
White 'horse tooth' maize	1·18
Fine oatmeal	1·18
Barley	1·25
Wholemeal flour	1·30
Oats	1·39
White flour	1·41
Wheat germ	1·54
Rolled oats	1·61
Wheat feed	1·69

7.4.2 *Insect Numbers* — It is possible to estimate from the carbon dioxide figure the numbers of certain insects which are present. The precision of the estimate depends upon the information available concerning the sample. Usually this will be limited to an indication of species of insect present (obtained from direct observation with or without sieving) and the moisture content of the grain. If only a few species are present and the moisture content of the grain is low, the following data may be used for making such an estimate:

In a sieved sample of grain (that is, one from which free-living insects have been removed) a one-percent carbon dioxide figure indicates an infestation of approximately one weevil larva (*Calandra-Sitophilus* sp.) per 650 grains or 55 per kilogram. This estimate is reliable if all ages of larvae are present in approximately equal proportions, which implies (under constant conditions) that egg-laying has proceeded at a constant rate up to the time of sampling. The various instars will then be present in numbers proportional to their duration. If there is reason to think that older stages predominate, a lower infestation than this is indicated while, if younger stages predominate, the infestation is higher. In Table 1, similar information is given for a number of other insects commonly infesting grain, at 25°C (standard) and some higher temperatures. This data may serve as a measure of the relative importance of the species listed in causing heating of grain.

TABLE 1 NUMBER OF INDIVIDUALS PER KILOGRAM OF GRAIN WHICH WILL PRODUCE A CARBON DIOXIDE (CO_2) FIGURE OF 1 PERCENT (TESTED ON WHEAT)

Insect	Number of Individuals per Kilogram at				
	25°C	28°C	30°C	33°C	37°C
Adults:					
Calandra (*Sitophilus*) sp.	40	31	26	24	33
Rhizopertha sp.	106	88	70	57	—
Oryzaephlus sp.	352	242	220	—	—
Laemophloeus sp.	462	333	—	—	—
Tribolium sp.	176	110	—	—	—
Larvae:					
Calandra, 1st stage*	352	220	—	—	—
Calandra, 2nd stage*	198	99	—	—	—
Calandra, 3rd stage*	88	53	—	—	—
Calandra, 4th stage*	18	15	—	—	—
Calandra, Pupae	110	88	—	—	—
Calandra, Mixed preadult stages	55	44	—	—	—
Ptinus tectus	106	—	—	—	—
Ephestia elutella, full grown	22	—	—	—	—
Ephestia elutella, half grown	29	—	—	—	—

*Instars change their carbon dioxide production during their development and the figures given refer to the mean carbon dioxide production by each instar. The fourth larval instar covers a wider range than the others, including a fall to a very low level (about 110 per kg per 1 percent carbon dioxide figure) in the pre-pupal stage.

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